

APPENDIX A

Table of Contents

	'624 Pending Spec.	'354 First Spec.
	<u>Page</u>	<u>Page</u>
Field of Invention	1	1
Summary of the Invention	35	20
Brief Description of the Figures	40	23
Detailed Description of the Invention	40	23
Examples	61	35
Example 1	64	37
1.1 Cloning the <i>Bacillus thuringiensis</i> Insecticidal Protein Gene . . .	64	37
1.2 Immunological Analysis	66	38
1.3 Sequence Analysis	67	38
Example 2	68	39
2.1 Introduction of BamHI site into the Insecticidal Protein Gene . . .	68	39
2.2 Construction and Modification of a Promoter Vehicle	70	40
2.3 Introduction of a Kanamycin Resistance Gene into pKS-proI (Bam)	72	41
2.4 Introduction of p11-83b into Ti-Plasmids	75	43
2.5 Cloning and Isolation of a Kanamycin Resistance Gene	75	44
Example 3	76	44
3.1 Moving the NOS Gene into M13 mp7. .	76	44
3.2 Placement of an NcoI site behind the NOS promoter	78	45
3.3 Moving the Insecticide Gene into M13mp8	79	45
3.4 Placement of an NcoI site at the Insecticide Gene Translation Start .	79	46
3.5 Assembly of a Plant Expressible Insecticide Gene in a Shuttle Vector	80	46
3.6 Insertion into TIP Plasmids, Plant Infection and Regeneration	81	47

Example 4	82	48
4.1 Moving the Phaseolin Gene into M13mp7.83	48	
4.2 Placement of an NcoI Site behind the Phaseolin Promoter	84	48
4.3 Placement of Hind III Site at the 3'-End of the Phaseolin Gene	85	49
4.4 Inserting the Insecticide Gene	87	50
4.5 Moving the Modified Phaseolin Gene Into a Shuttle Vector	87	50
4.6 Insertion into TIP Plasmids, Plant Infection and Regeneration	88	51
Example 5	88	51
5.1 Infection with Hairy Roots	89	51
5.2 Regeneration of Roots to Plants	89	52
5.3 Use of Non-Hairy Root Vectors	90	52
Example 6	90	52
6.1 Infection with Crown Gall	90	52
6.2 Culture of Transformed Tissue	91	53
6.3 Regeneration of Plants	93	54
6.4 Vectors Used	93	54
Example 7	94	54
7.1 Binding Antibody to Plates	94	54
7.2 Tissue Homogenation	95	55
7.3 Binding Enzyme	95	55
7.4 Assay	96	55
Example 8	96	56
Example 9	97	56
Example 10	98	57
10.1 Oligonucleotide Synthesis	98	57
10.2 Use for Oligonucleotides	99	57
10.3 Oligonucleotide-Directed Mutagenesis.	99	57
Example 11	101
11.1 Cloning of an Insecticide Gene 3'-end	102

11.2 Construction of a Full-Length Insecticide Gen	102
Example 12	103
12.1 Preparation of pH400, a Sub-Ti Plasmid	104
12.2 Preparation of pDOB513	106
12.3 Preparation of T-DNA ORF24 Transcription Controlling Sequences.107	
12.4 Preparation of the Insecticide Gene. 113	
12.5 Combination of an Insecticidal Protein Structural Gene with ORF24 Transcription Controlling Sequences. 114	
12.6 Insertion of Plant-Expressible Insecticide Gene Into A Sub-Ti Plasmid . . . 115	
12.7 Plant Transformation	115
12.8 Expression in Plant Tissue: Immunoassays	116
12.9 Expression in Plant Tissue:Bioassays.121	
Example 13	125
13.1 Plant Transformation Vectors	125
13.2 Modification of an Insecticide Gene's 5'-End	126
13.3 Modification of an Insecticide Gene's 3'-End	128
13.4 Fusion of an Insecticide Gene with NPT2 Sequences	129
13.5 Construction of Transcription Vectors.130	
13.6 Assembly of Direct Transformation Vectors	132
13.7 Fusion of an Insecticide Gene with Hygromycin Sequences	133
13.8 Description of Binary Vectors	134
13.9 Deposited Strains	139
Example 14	140
14.1 Maize Protoplast Transformation . . . 140	
14.2 Assay of Insecticidal Protein	143
Example 15	144
15.1 Leaf Tissue Transformation	144



15.2 Hypocotyl Transformation	146
15.3 Transformation with Agrobacterium Strains	147
15.4 ELISAs	148
15.5 Western Blots	149
15.6 Bioassays	152
15.7 Nucleic Acid Analysis	153
15.8 Results	153
Example 16: Potato Transformation Procedure.	157
16.1 Plant Material	157
16.2 Bacterial Strains	157
16.3 Tissue Culture Media	158
16.4 Procedure	158
16.5 Results	159
Example 17: Cotton Transformation.	160
Table 1. Insects susceptible to <i>B. thuringiensis</i> insecticidal protein	163
Table 2. Plants recommended for protection by <i>B. thuringiensis</i> insecticidal protein	178
Table 3. Varieties of <i>B. thuringiensis</i> .	179
Table 4. Index of plasmids and strains .	180
Table 5. Deposited strains	185
Table 6. MS Medium	186
Table 7.	187
Table 8.	188
Table 9.	189
Table 10.	190
Table 11.	191
Table 12. Transformation of <i>Lycopersicum esculentum</i> Hypocotyl and Leaf Disc Tissues with Various Binary Vectors	192
Table 13. Summary of Bioassays and ELISAs on Tomato	193